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Research Article

The Absorption and Distribution of Heavy Metals of Dominant Plant for Ecological Restoration of Stone Coal Mine

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The exposed slopes formed by stone mining had caused serious problems of environmental pollution. The ecological restoration was a very effective measure to solve it. In this study, the *Indigofera amblyantha* Craib was the dominant species in the ecorestoration during the first two years of ecological restoration, which was based on analyzing the importance value (IV) and diversity indexes of plant species. Meanwhile, the ecorestoration process helps slow down the increase of the concentrations of some heavy metals in the substrate material; however, the changing trends of these metals were not uniform. The root and leaf were the main parts of heavy metal distribution, respectively, corresponding to Pb, Cr, As, Sb, and Ni in root and Cu, Mn, and V in leaf. It confirmed that *Indigofera amblyantha* Craib had an advantage ability to enrich and transfer Cd, Cu, and Mn obtained from their biological concentration factor (BCF) and biological transfer factor (BTF). The Sb, Cd, and V were the main factors affecting the IV by the redundancy analysis. These fine characters of *Indigofera amblyantha* Craib help explain it well adapted to the ecorestoration of stone coal mines. The current results are valuable to evaluate and extend the application in ecorestoration engineering of mining areas and other heavy metal-contaminated sites.

1. Introduction

Stone coal is a kind of coal resource stored in ancient stratum, with the characteristics of a low-carbon (10-25%), low calorific, high ash (65-80%), and high-sulfur (2-5%) [1, 2], and associated with polymetallic composition, such as V, Mo, Ni, Cu, Pb, Cd, Cr, Fe, and Zn, and their compounds, etc. [3, 4]. Stone coal is an important V resource [2, 5]. Then, the increasing exploitation of stone coal mines led to form a lot of bare slopes and waste piles exposing to the environment [6, 7]. Many exposed high escarpments derived from the mining of stone coal were exposed to atmospheric oxygen, which resulted in acidic mine discharge (AMD) formed from rain [8, 9]. The AMD could dissolve heavy metals discharged into the water, such as V, Ni, Cd, Au, Ag, Cu, and Zn [10, 11]. For example, for the fine particle sizes of stone coal, the dissolved rates of As, Pb, V, Cd, and Cr were 2.96%, 0.95%, 0.35%, 0.25%, and 0.01% after 100 days in pH 4.5 solution, respectively [12]. These heavy metals further hazarded surrounding farmland and rivers. Thus, it was worthy of close attention and needs an effective measure to solve it.

The ecorestoration was a very effective measure to reduce the ecological environment risk of the exposed slopes formed after the stone coal mining [13–15]. At present, covering soil slope and growing plants was mainly ecological restoration methods [16–18]. However, different from the conventional ecorestoration, the dry, innutritious, and heavy metal stress was the most limiting factor on these sites containing heavy metals for plant establishment [19]. Therefore, providing a suitable environment for plants to grow and selecting tolerant plant species were essential to ecological restoration. However, there was a lack of research reports on ecorestoration and the plants adapted in exposed slopes of the stone coal mines.

In this work, we firstly found *Indigofera amblyantha Craib* growing very well in ecorestoration engineering of the stone coal mine in Yiyang, Hunan province. *Indigofera* *amblyantha* Craib, with the characteristics of drought resistance and barren tolerance, has been successfully applied to soil-water conservation and slope restoration. However, there is a lack of research on the tolerance of *Indigofera amblyantha* Craib to heavy metal-contaminated sites. Ten heavy metals, Pb, Cd, Cr, As, Cu, Zn, Ni, Sb, Mn, and V, in root, leaf, stem, and stem-xylem of *Indigofera amblyantha* Craib were measured to determine where the part was conducive to heavy metal enrichment. The biological concentration factor (BCF) and biological transfer factor (BTF) further analyzed the advantage ability to enrich and transfer heavy metals. The relationship between the growth of *Indigofera amblyantha* Craib and heavy metal distribution in soil is very important to understand the tolerance of heavy metals. As a result, these findings will help guide the ecorestoration engineering of the stone coal mine.

2. Materials and Methods

2.1. Study Sites. The ecological restoration engineering of the exposed slope of the stone coal mine was in Nijiang Kou, Yiyang City, Hunan province, where it belongs to the subtropical humid continental monsoon climate. The annual average temperature was 16.1-16.9°C, the annual sunshine hours were 1348-1772 with the annual cumulative rainfall of 1230-1700 mm, and the frost-free period was 263-267 days. The total area of the ecological restoration engineering was about 75,000 m^2, and the construction was completed in March 2019. In this study, about 3,000 m^2 as the sample sites was selected to future analyze the changes in the recovery process due to some areas which were not conducive to sampling. The average slope ratio of the selected area was 1 : 0.25, and the average sea allocation was about 70-80 m. To understand the environmental background of the area, the heavy metal content in stone coal was measured.

2.2. Ecological Engineering. The ecological restoration engineering adopted the thick layer substrate material spraying after hanging net for vegetation restoration [20]. Firstly, the galvanized wire mesh was fastened to the stone slope by the rock bolts in order to make the soil substrate material better attached to the slope. Meantime, adding herbaceous fibers to the net at the spacing of 1 m was to prevent the substrate material layer down and provide support for plants' root growth. Second, the substrate material was sprayed onto the rocky slope with wire mesh by a compressed-air spraying machine and rotor concrete conveyer (5 m^3 h^{-1}, 0.12 MPa, Hunan Changde Universal Compressor Co., Ltd., Changde China). There was no seed added into the substrate material for the first spraying at the thickness of 6 cm, and following the second spraying mixed with the plant seeds with the thickness of the layer was about 4 cm. The total thickness of the layer was 8-10 cm. Third, nonwoven fabric was covered in the surface of the soil layer after spraying to reduce the evaporation of water and soil erosion from the slope surface and protect the seeds washed away by rain.

The substrate material and seeds were two important components of ecological restoration engineering. The substrate material was composed of a variety of material mixing, including soil, peat soil, sawdust, organic fertilizer, compound fertilizer, aggregate agent, water retention agent, biobacterial manure, and seeds according to a certain proportion. The composition requirements and functions of substrate material are detailed in Table S1 and S2, Supplementary Information (SI). Plants were the key to rocky slope ecoengineering. Therefore, the characteristics of drought and barren tolerant of plants were selected to use in the side slope ecological restoration. The selection of plant species was based on reports in successful engineering from common rock slope and similar mine restoration [21–23], such as *Indigofera amblyantha* Craib and *Robinia pseudoacacia* with strong resistance to adversity [24–26]. Table S2 lists the selected plant species used well applied in rocky slope ecoengineering. However, it was not clear whether these plants were tolerant to heavy metals for rocky slope ecoengineering in the stone coal mine. The expected density of species was based on references and empirical values (Table S2 and its description in detail).

2.3. Sample Collection and Analysis. The investigation time lasted for two years, and the sampling time was at the 3, 6, 9, 15, 18, and 21 months after ecorestoration (i.e., June, September, and December 2019 and June, September, and December 2020, respectively). Twelve ecological investigation samples were established at 5 m away from the bottom and top of the slope and middle of slope in four different locations, assessing plant species and communities in ecoengineering restoration. In the investigation samples, $3 \times 3\ m^2$ quadrats were used for the shrubs, and internal $1 \times 1\ m^2$ was chosen to determine the herb layer. The number, coverage/crown width, and height of each species in the ecoengineering communities were measured in these quadrats. The importance value (IV), Shannon-Wiener index (H'), and Simpson index (D) were used to represent the vegetation diversity, respectively [27–29], and their description in detail is shown on Text S2 in SI. The importance value (IV) as a comprehensive index can reflect the position and function of a species in a given community. Thus, the higher IV showed the species with better adaptability in the environment [30].

To prevent the sampling of the substrate material and dominant plants from affecting the growth of the plants in ecological investigation samples, dominant species accompanying the substrate material of different recovered times were sampling in the adjacent area outside the ecological investigation samples. All substrate material and plant samples were sealed with polythene bags and transported to the laboratory. The substrate material soil samples air-dried were sieved through a 100-mesh sieve and used to further analyze the content of the heavy metal. After washing carefully with deionized water, the plant samples (root, leaf, stem xylem, stem phloem, and fruit/seed) were oven-dried at 80°C until dry-weight no longer changes [31, 32] and further analyzed the content of heavy metal in parts of plants.

The content of heavy metals, including Pb, Cd, Cr, As, Cu, Zn, Ni, Sb, Mn, and V in stone coal, artificial soil, and plants, were measured by ICP-MS (NexION 350X, PerkinElmer, USA) after digesting tissues following the Chinese National Standards for Food Safety Determination of multiple elements (GB5009. 268-2016). The 0.10 g collected samples were added to the 50 mL Teflon crucibles and added 5 mL deionized water. Then, 5 mL hydrochloric acid, 5 mL nitric acid, 3 mL hydrofluoric acid, and 2 mL perchloric acid were added into the crucibles in turn, respectively. The crucibles covering lid were placed on the electric heating plate and heated 0.5 h in the 120°C and continue to heat 0.5 h in the 150°C. Then, open the lid and heat it until it is nearly dry to drive out the acid at 180°C. After cooling, 2 mL nitric acid-soluble was added to dissolve again, joined in ultrapure water to 50 mL volumetric, and then filtered through 0.22 μm membrane filtration [33]. The solution after filtration further analyzed the content of heavy metals.

The biological concentration factor (BCF) and biological transfer factor (BTF) indexes were calculated as follows [34, 35]:

$$\mathrm{BCF} = \frac{\text{heavy metal content in plants}}{\text{heavy metal content in soils}} \times 100\%, \quad (1)$$

$$\mathrm{BTF} = \frac{\text{heavy metal content in overground parts}}{\text{heavy metal content in root}} \times 100\%. \quad (2)$$

The redundancy analysis (RDA) was used to analyze the plant species response to the factors that had a greater impact on growth. The result of RDA could find out the positive or negative factors on the ecorestoration process. The RAD was performed with CANOCO 5.0 [36]. When the angle between different environmental factors and/or species was acute, it indicated a positive correlation and on the contrary obtuse represented a negative correlation between them [37–39].

3. Result and Discussion

3.1. Plant Community Change. The time-dependent pictures of the actual recovery are shown in Figure 1. It was obvious that the vegetation coverage of the slope has been significantly improved and remained a high coverage after 21 months. The analysis of the growth of species and changes of the community help to clearly understand the driving processes. The composition and individual amount of species were the most important factors affecting the ecorestoration community. From the analysis of the results (Figure 2), the *Carex siderosticta* Hance and *Indigofera amblyantha* Craib were the dominant species for the herbs and fruticose community, respectively. The IV of species in the herbaceous community was *Carex siderosticta* Hance>-*Lolium perenne* L.>*Cynodon dactylon* (L.) Pers. in order (Figure 2). The *Phytolacca acinosa Roxb* and *Erigeron acer* Linn. were exotic local species and were less impact on the community. The IV of *Carex siderosticta* Hance showed a fluctuating trend and with an obvious downward trend in winter (recovered time at 9th and 21st month); however, the cold season *Lolium perenne* showed increasing trends in winter. The IV of *Cynodon dactylon* (L.) Pers. showed no significant change over time. For IV of species in the fruticose community, there has a reduced trend in turn: *Indigofera amblyantha* Craib>*Crotalaria pallida Ait.*>*Cassia surattensis* Burm.>*Robinia pseudoacacia L.*>*Rhus chinensis* Mill. in different recovery times (Figure 2). The *Indigofera amblyantha* Craib was the dominant species among these species, of which the IV showed an increasing trend. The IV of species *Crotalaria pallida Ait.* in the second year showed a slight decline, probably due to its poor drought tolerance. In addition, for the top, middle, and bottom of slope positions, the IV of species did not differ significantly in different positions (Figure S1). The reason may be the height difference of samples was not obvious enough (30-50 m).

The diversity index of the plant community was an important indicator, which can quantitatively reflect the stability of the community. The higher values indicated that the ecorestoration community was in better condition. The Ma, H' and D indexes of the herbosa and fruticose community consistently showed upward trends overall (Figure S2). However, the Ma, H', and D indexes of the herbs showed down slightly in the second spring, i.e., from 9th to 15th months. There was no significant difference in diversity indexes between top, middle, and bottom of slope positions.

The dominant species *Indigofera amblyantha* Craib was firstly found that well adapted to the ecorestoration of stone coal mines, which could grow well in poor and pH 4.5-7.5 soil due to the characteristics of resistance to infertility [40, 41], a strong drought tolerance [42, 43], and strong tensile strength of root. Thus, these characteristics of *Indigofera amblyantha* Craib determined its dominant position under adverse circumstances. The fruticose community was in a dominant position relative to herbosa community, and they played more greater role in the process of vegetation restoration and stabilization. Thus, the distribution of heavy metals of dominant plant in fruticose community was further analyzed.

3.2. Heavy Metals in the Substrate Material. In order to further investigate the heavy metal changes in the ecorestoration, the concentration of heavy metals in stone coal and substrate material with recovered time was measured (Figure 3). Except for Mn, heavy metals of stone coal (s.coal) were higher than those in the initial substrate material (init.), especially Cd, Cr, Cu, Ni, and V were more obvious. Despite the bare stone coal has been covered by a layer of replacing with the artificial substrate material, there had still heavy metals leaching from stone coal under the action of water and oxygen. In other words, stone coal was an important source of heavy metals in the presence of rain and oxygen [8, 44]. It is worth noting that the Mn in the initial artificial soil matrix (init.) was higher than in the whole recovery period. And the concentration Mn was a downward trend from the initial to the 18th month and then increased again. The possible reason was that the initial addition of quick-acting fertilizers contained high levels of Mn, but it lost heavily in early recovery time.

The changing trend of Pb, Cu, Sb, and V in substrate material shown firstly raised and then fell. The results suggested that these heavy metals were leached from stone coal

Before recovered

(a) 2018.11

Eco-engineering restoration completed

(b) 2019.3

The 3rd month after restoration

(c) 2019.6

The 9th month after restoration

(d) 2019.12

The 18th month after restoration

(e) 2020.9

The 21st month after restoration

(f) 2020.12

Figure 1: The time-dependent ecological restoration process of stone mine slope.

into substrate material in the early stages of ecological restoration. After 6 months of ecological restoration, the concentration of Cu, Sb, V, and Pb decreased significantly, which may be related to the increase in vegetation coverage. The concentration of Sb decreased was more obvious in the second year (8.01 mg kg^{-1} at twenty-first month) relative to the highest point (28.11 mg kg^{-1} at the 6th month). In addition, as a special associated heavy metal in stone coal, the concentration of V in stone coal (1636.53 mg kg^{-1}) was significantly higher than that of the initial substrate material (84.45 mg kg^{-1}), its concentration was rising until the 6th month and then falling to a steady concentration (897.04 mg kg^{-1}). The Zn, Ni, and Cd in substrate material were on the increasing trend overall. The concentrations of Zn in the substrate material had little change overall in recovered time. In contrast, the concentrations of Cd and Ni in the substrate material were generally upward overall. In addition, the concentration of Cd and Ni in the substrate material increased 5.71 and 2.23 times, respectively, from 0.14 to 0.80 mg kg^{-1} and 23.52 to 52.46 mg kg^{-1}. The high concentration of these metals in stone coal might be one of the driving factors for the increase of what was in the substrate material. The concentrations of Cr and As in the substrate material were slightly rising in the initial stage of ecorestoration and then showed a downward trend. However, the concentrations of Cr and As had a slight upward in the twenty-first month. These changes of heavy metals in substrate material were closely related to plant absorbing and transferring.

3.3. Distribution of Heavy Metals in Indigofera amblyantha Craib. As the dominant species, the ability of *Indigofera amblyantha* Craib to tolerate, accumulate, and migration of heavy metals was an important factor to explain its

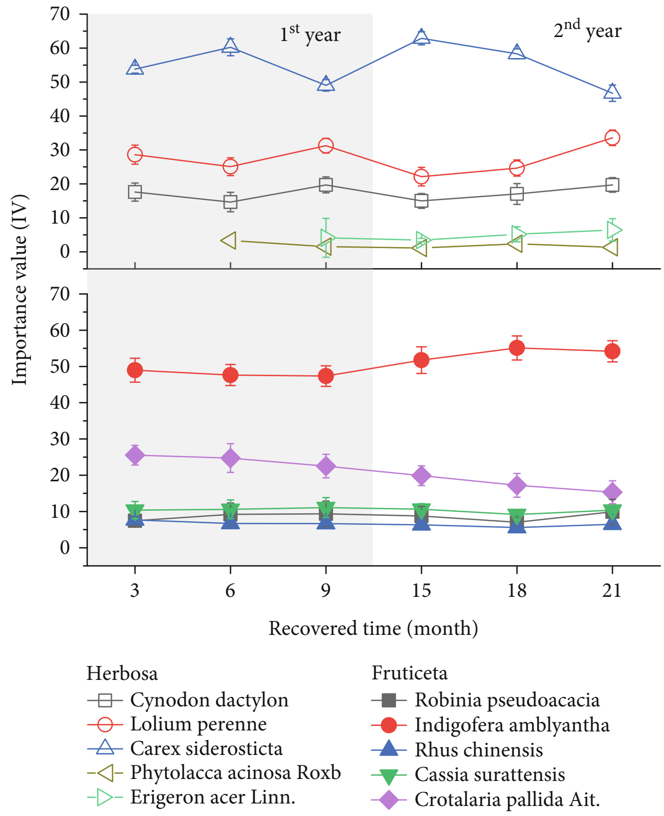
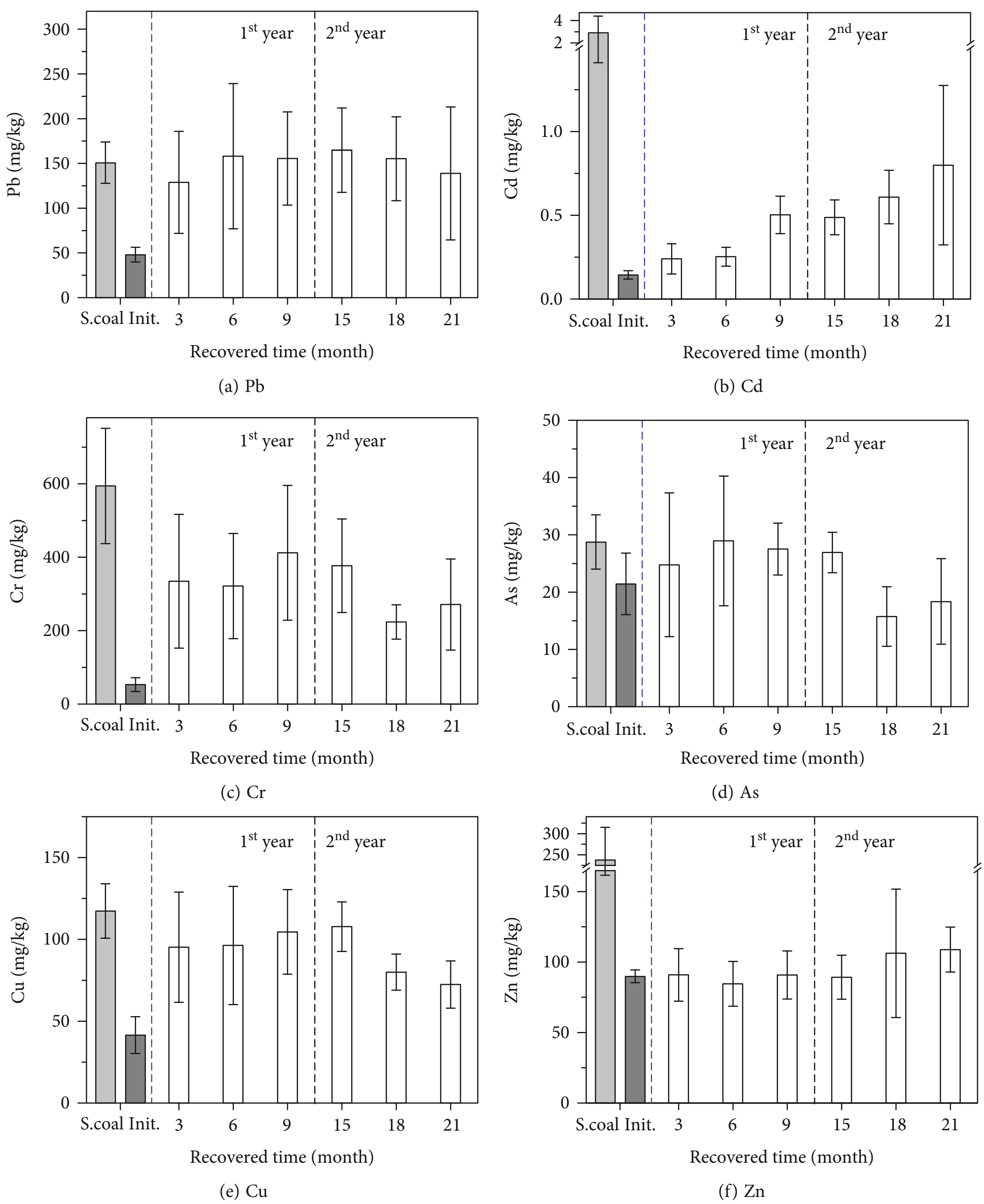


Figure 2: The importance value (IV) of species in herbosa and fruticeta community with the recovered times. The error bar was the standard error.

adaptability in the ecorestoration of stone mine slope. The distribution of heavy metals in *Indigofera amblyantha* Craib is shown in Figure 4. Compared to the control group at twenty-first month (CK(21)), the concentrations of As in leaf, Cd, Cu, Zn, and V in the root, and Ni in stem-xylem were approximately equal to the CK(21) group, which suggested that these parts were not affected by those heavy metals. The concentrations of different heavy metals in roots, stems, and leaves varied greatly (Figure 4). By the analysis of the ratio of heavy metals in different organs of the experimental group (recovered the 21st month) and CK(21), the Cd, Zn, and V of stem-phloem, Pb and As in stem-xylem, Cr, Cu, Ni, and Mn in leaf, and Sb in root were higher than that of other organs (Table S3). The concentrations of Cd, Zn, and V in stem-xylem were 1.88, 2.74, and 1.71 times, Pb and As in stem-phloem were 4.18 and 4.28 times, Cr, Cu, Ni, and Mn in leaf were 263, 1.94, 2.78, and 3.62 times, and Sb in root was 2.11 times that of the CK groups after 21 months in ecorestoration, respectively. The result showed that the accumulation of heavy metals in plant organs in contaminated sites has increased.

For the changes of concentration with recovered time, except Cd, Cu, and Sb, the average concentrations in roots, stems, and leaves increased gradually with the recovered time increasing in the overall trend, and the rate of increase in the second year was higher than that in the first year. Different from other heavy metals, Cu decreased in the first year, but increased gradually in the second year, and Sb showed the rule of ascending-descending-ascending in stem, leaf, and root. Except for V and Sb, the changes of concentrations of other heavy metals in the stem-xylem were not significant. The concentrations and increase ranges of Pb, Cr, As, and Ni in root, Mn and V in leaf, and Zn in stem-phloem were the highest, respectively. It is worth noting that the concentration of Cd was the highest in the first 3 months and then decreased gradually, which may be related to its strong migration. The concentration of heavy metals in pods of *Indigofera amblyantha* Craib was also different. The concentration of Cd, Zn, and Mn in the pod of the second year was lower than that of the first year, and other heavy metals were higher than that of the first year (Figure 5).

3.4. BCF and BTF of Heavy Metals in Indigofera amblyantha Craib. The concentration level could not reflect the tolerance and accumulation of heavy metals in plants. Thus, the biological concentration factor (BCF) and biological transfer factor (BTF) of heavy metals in different tissues of *Indigofera amblyantha* Craib were calculated to further analyze the characteristics of enrichment and migration of heavy metals (Figure 6). In general, the BCF > 1 is one of the standards for heavy metal enrichment plants, and the BCF and BTF > 0.5 can be used to adsorb soil heavy metals in practical application [45, 46]. The BCF of heavy metals in organs of *Indigofera amblyantha* Craib increased with the recovered time, in order as follows: leaf>stem-phloem>root>stem-xylem.



(a) Pb (b) Cd (c) Cr (d) As (e) Cu (f) Zn

Figure 3: Continued.

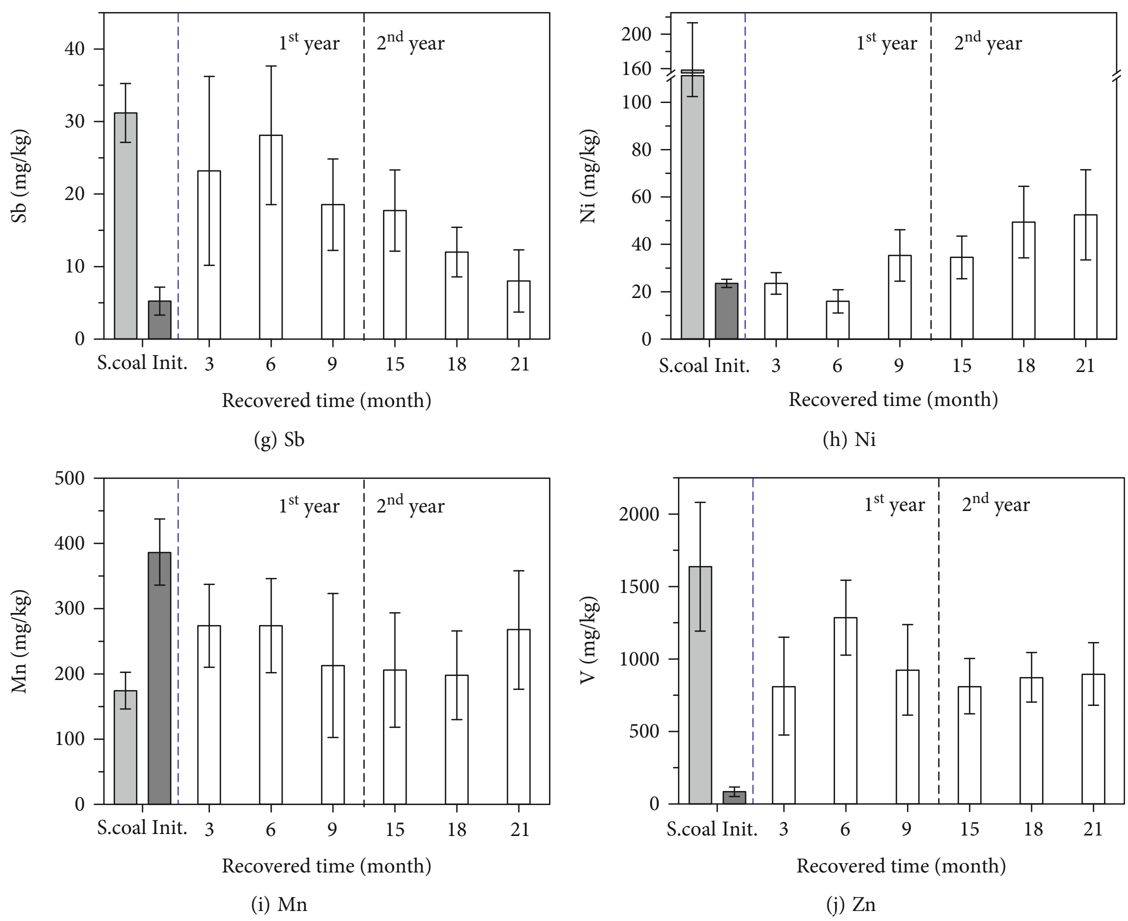


Figure 3: The concentrations of heavy metal of both stone coal and substrate material with recovered time. The s.coal and init. represented stone coal and substrate material, respectively. The error bar was the standard error.

However, the BCF heavy metal in the phloem was higher than that in the xylem; thus, heavy metals were relatively easy to accumulate in the phloem instead of the xylem. The BCFs of Cd, Cu, Zn, and Mn were higher than other heavy metals in each recovery time. The BCFs of Cd, Mn, and Cu in leaf were 0.905, 0.891, and 0.515 after recovered 21 months, respectively. In addition, the BCFs of Cd and Zn in stem-phloem were 0.697 and 0.478, respectively. There indicated that *Indigofera amblyantha* Craib has a stronger ability to accumulate Cd, Mn, Cu, and Zn, especially in the leaf and stem-phloem. However, the BCF does not reflect the ability of organs to transport heavy metals; thus, the BTF be used to evaluate the ability to transport and enrich heavy metals from underground to surface. The BTF of Cd, Cu, Zn, Ni, Sb, and Mn in stem or leaf was higher than 1, and the BTF of Zn was even 3.16 in the stem-phloem at 18 months, and Cd, Cu, and Mn values were up to 1.92, 3.48, and 4.27 in the leaf at 21 months, especially. Like the BCF, the BTF of most heavy metals in the leaf was the highest, and the BTF of heavy metals in the phloem was higher than that in the xylem. The BTFs of Cu and Mn in leaf, Zn in stem-phloem, and Cd in both leaf and stem-phloem were higher than in other parts of organs. These heavy metals may be transported from the xylem to the leaves and then accumulated in the phloem. But, the BTF of Zn in stem-phloem and leaf was a significant drop in winter (9^{th} and 21^{st} months). There indicated that *Indigofera amblyantha* Craib had a better ability to enrich and transfer Cd, Cu, Zn, and Mn. After being absorbed by roots in the soil, Cd, Cu, and Mn were mainly transferred to leaves, while Zn was mainly accumulated in stem-phloem and roots. However, the enrichment and transferability of Pb, Sb, V, and Cr were low. It suggested that *Indigofera amblyantha* Craib has good tolerance to Pb, Sb, V, and Cr.

3.5. Response to Heavy Metals in Substrate Material. The response of dominant species *Indigofera amblyantha* Craib to heavy metals in substrate material was analyzed by the redundancy analysis (RDA). The RDA could identify the factors with a greater impact on plant growth [36, 38]. The longer the radiation of environmental factor, the greater influence of the factor has. The RDA analysis between IV of plant species and heavy metals is shown in Figure S3. The first two axes explained 76.4% of the microbial community variation (axis 1: 41.0% and axis 2: 35.4%). The total variation was 0.841, and $P = 0.082$. The Sb, Cd, and V in the substrate material were the main factors affecting the IV of plant species (Figure S3). The IV of *Indigofera amblyantha* Craib was a positive correlation with V and Sb and a negative correlation with Cd.

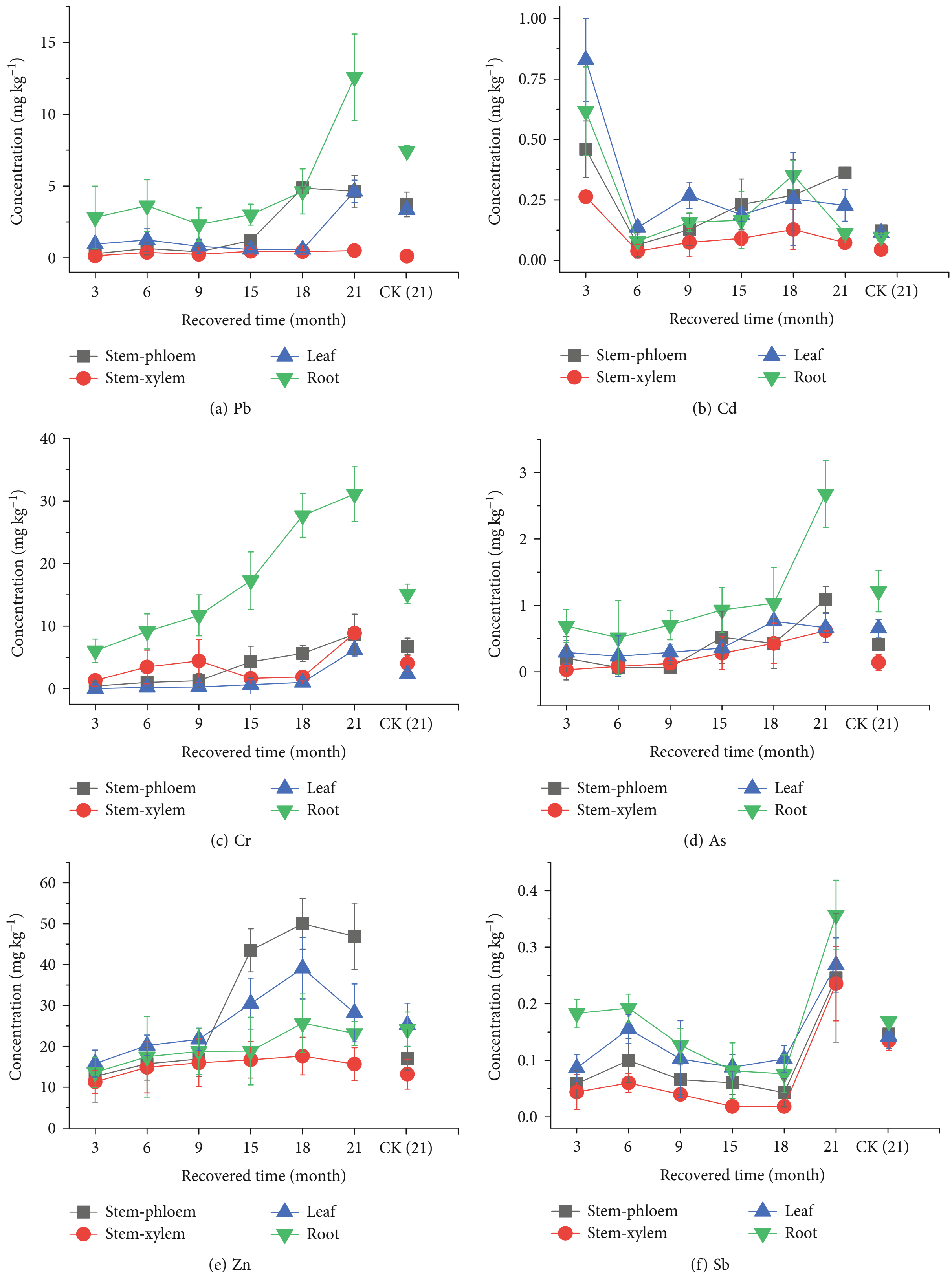


Figure 4: Continued.

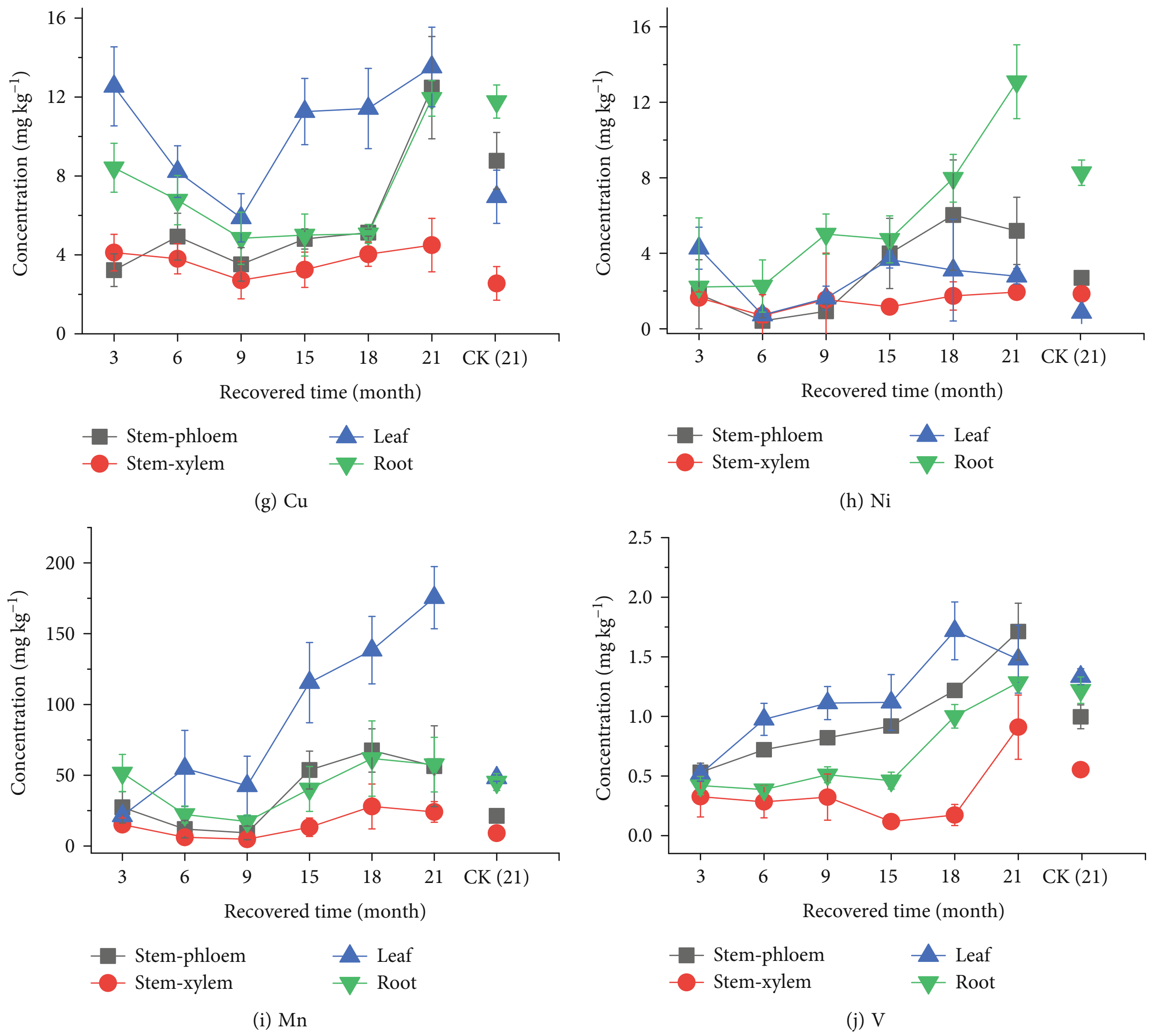


FIGURE 4: Effect of recovery time on contents of heavy metal in different organs of *Indigofera amblyantha* Craib. The CK(21) represents the control group at twenty-first month. The error bar was the standard error.

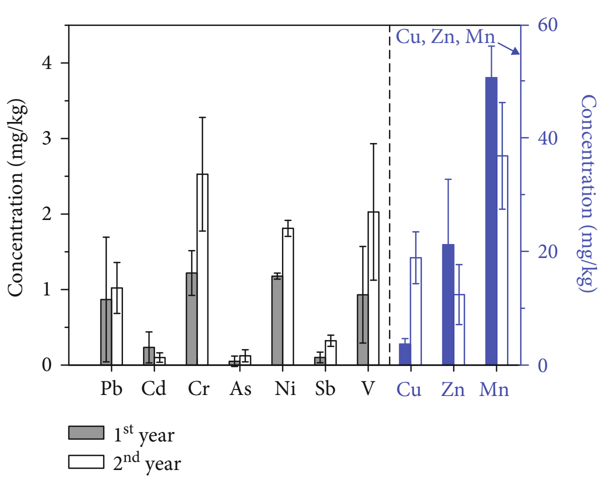


FIGURE 5: The concentration of heavy metal in fruit of *Indigofera amblyantha* Craib in two natural years. The 1st and 2nd years were the ninth month and the twenty-first month, in winter. The error bar was the standard error.

Indigofera amblyantha Craib was also used for ecological restoration of tungsten mine site, and the absorption of Cu and Zn was relatively significant through the analysis of heavy metals in the soil, which confirmed that *Indigofera amblyantha* Craib had a good capacity of accumulation and transport of Cu and Zn from stone mines in this study [47]. Chen et al. suggested that *Indigofera amblyantha* Craib had moderate tolerance and bioaccumulation capability for Pb and Cd [48, 49]. However, there was no advantage of *Indigofera amblyantha* Craib on accumulation, transport, and tolerance to Pb was found in this study. Besides, there were no other reports related to heavy metals in *Indigofera amblyantha* Craib. Although *Indigofera amblyantha* Craib does not meet the standard of heavy metal super accumulation in plant, it still can been used for the ecological restoration of soil contaminated by Cd, Cu, Zn, and Mn or mining areas due to its strong enrichment and transport capacity for Cd, Cu, Zn, and Mn and its large biomass.

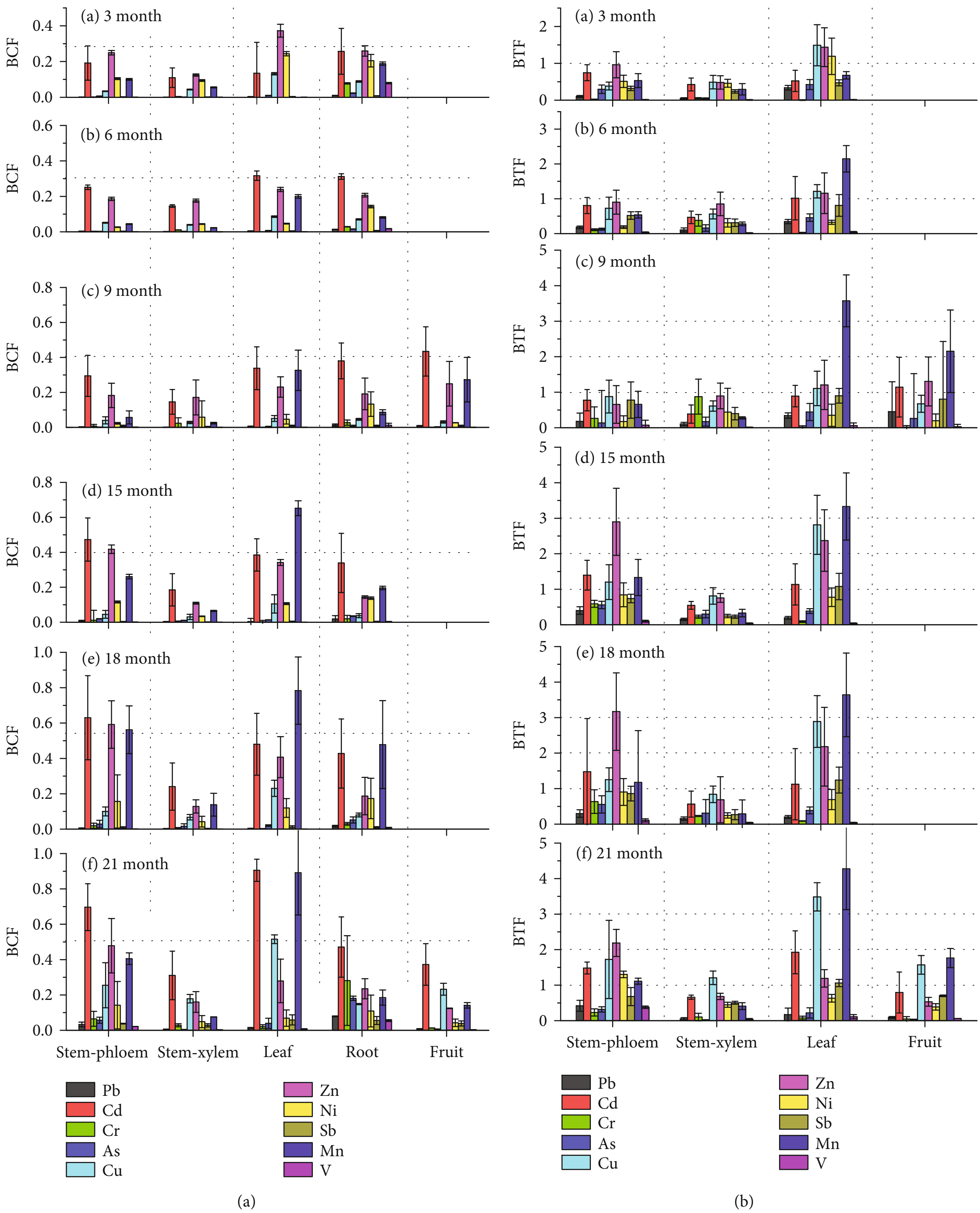


Figure 6: The (a) BCF and (b) BTF of heavy metal in different organs of *Indigofera amblyantha* Craib. The error bar was the standard error.

4. Conclusions

In this study, we firstly found that the *Indigofera amblyantha* Craib showed great adaptability based on two years of follow-up investigation on ecorestoration engineering in expose slopes of the stone coal mines. Specifically, the *Indigofera amblyantha* Craib was confirmed to be the dominant species in the ecorestoration based on the IV and diversity indexes obtained from the investigation of number, height, crown width, or coverage of species. The changes of concentrations of heavy metals (Pb, Cd, Cr, As, Cu, Zn, Ni, Sb, Mn, and V) in substrate material suggested that they were greatly affected by the stone coal parent material and ecorestoration effect. Further, the concentrations and BCF and BTF indexes of heavy metals in leaf, stem, root, and fruit of dominant species confirmed that *Indigofera amblyantha* Craib had a better ability to enrich and transfer Cd, Cu, Zn, and Mn and has good tolerance to Pb, Sb, V, and Cr. Overall, the results of this study could shed light on the applicability of *Indigofera amblyantha* Craib in ecorestoration engineering of mining areas.

Data Availability

All data generated or analyzed during this study are included in this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary data associated with this article can be found, in the online version. *(Supplementary Materials)*

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